

CLAIMS

- 1 Universal primers named as 'mcb 398' and 'mcb 869' capable of amplifying a fragment of cytochrome b gene of any animal species in polymerase chain reaction (PCR) and revealing the identity of the biological material of any animal of unknown origin at species and sub-species level, said primers, having the sequences:

primers name	Sequence (5'-3')
mcb 398	"TACCATGAGGACAAATATCATTCTG"
mcb 869	"CCTCCTAGTTTGTAGGGATTGATCG"

2. Primers as claimed in claim 1 wherein the fragment of mitochondrial cytochrome b gene is capable of significantly discriminating amongst various evolutionary lineages of different animal species.
3. Primers as claimed in claim 1 wherein the fragment of mitochondrial cytochrome b gene is flanked by the highly conserved sequences amongst a vast range of animal species.
4. Primers as claimed in claim 1 wherein the fragment on mitochondrial cytochrome b gene which is polymorphic inter-specifically, but monomorphic at intra species sources.
5. Primers as claimed in claim 1 wherein in *Antelope cervicapra* species, the sequences of the fragment mentioned under claim 1 are as follows:

Mitochondrial cytochrome b gene sequence (398-869 bp) of *Antelope cervicapra*:

"taccatgaggacaaatcttttgaggagcaacagtcacccaatctccttcagcaatcccatacatcggtacaacctagtagaatgaatctgaggagggttctcagtagataaagcaacccttacccgattttgccttcacatttatcctccattatcattgacgcccttaccatagtagacactactgtttctccacgaacaggatccaacaaccccacaggaaatctcagacgcagacaaaattccattccacccctactacactatcaagatatcctaggagctctactattaatttaaccctcatgcttctagtcctattctaccggacctgcttgagaccagacaaactatacaccagcaaacccacttaatacacccccacatcaagcccgaaatgatacttctattgcatacgcaatcctccgatcaattcctaacaactaggagg"

6. A method for the identification of the animal from a biological sample, said method comprising the steps of:
- isolating and amplifying the DNA from the biological sample to be tested using the primers as claimed in claim 1,
 - sequencing the amplified products,

- c) blasting the sequence resolved in step (b) against mito database of National Centre for Biotechnology Information (NCBI) using BLAST program and determining the most likely family of the animal source of the biological sample,
 - d) blasting the sequence resolved in step (b) against non-redundant (nr) database of National Centre for Biotechnology Information (NCBI) using BLAST program and determining the most likely genus, species or more precisely the sub-species of the animal source of the biological sample,
 - e) identifying the most significant alignment of the sequence resolved with cytochrome b gene sequence of the animal identified in steps (c) and (d) respectively and selection of these animals as 'reference animals' for further studies,
 - f) isolating and amplifying and sequencing the DNA sequences from the reference animal on both strands in triplicate using the primers as claimed in claim 1,
 - g) aligning the sequences obtained using CLUSTRAL program and identifying the variable sites amongst the animals analyzed,
 - h) comparing the nucleotide sequences pair-wise to determine the variation among the animals resolved and identifying the nucleotide sequence to which the DNA sequence of the biological sample bears maximum similarity as the source animal of the biological sample.
7. A method as claimed in claim 6 wherein the universal PCR protocol works universally with the DNA template of any unknown animal origin and the universal primers mentioned under column 4.
 8. A method as claimed in claim 6 wherein the Amplification reactions should be carried out in 20 μ l reaction volume containing approximately 20 ng of template DNA, 100 μ m each of dNTPs, 1.25 pmole of each primer, 1.5mM MgCl₂, 0.5 unit of AmpliTaq Gold (Perkin-Elmer-Cetus, USA) DNA polymerase and 1X PCR buffer (10mM Tris-HCl, pH 8.3, and 50mM KCl). The amplification profiles followed should be: an initial denaturation at 95°C for 10 min, followed by 35 cycles each of denaturation at 95°C for 45 s, annealing at 51°C for 1 min, and extension at 72°C for 2 min. The extension step at 35th cycles should be held for 10 min.
 9. A method as claimed in claim 6 wherein the method enables identification of species of analyzed material (i.e. the DNA isolated from confiscated animal remain of unknown origin) using the public databases such as GenBank, NCBI etc.

10. A method as claimed in claim 6 wherein the method is used for animal identification to establish the crime with the criminal beyond a reasonably doubt.
11. A method as claimed in claim 6 wherein the method is used to establish the identity of biological materials such as skin, horns etc confiscated from animal poachers, if it is that of an endangered species.
12. A method as claimed in claim 6 wherein the method is used for establishment of the identity of confiscated animal parts and products of endangered animal species for the purpose of production of molecular evidence of animal hunting and related crime in the court of law, so that the human violation of the wildlife resources could be controlled.
13. A method as claimed in claim 6 wherein the method is used to have an idea of the geographical location of the commitment of wildlife crime based on the cytochrome b gene haplotype of poached animal identified by the universal primer invented.
14. A method as claimed in claim 6 wherein the method is used for animal identification to detect the adulteration of animal meat in food products for the purpose of food fortification, by the food fortification agencies.
15. A method as claimed in claim 6 wherein the method is used to provide a universal technique for detection of the origin of blood or blood stains etc collected from the scene of crime related to offences such as murder, rape etc, in order to establish the origin of blood found at scene of crime when it sounds as if criminals have wontedly spread the blood of an animal at the scene of crime, to confuse the crime investigation agencies and forensic scientists with human blood.
16. A method as claimed in claim 6 wherein the method is used so that it can be converted to a (a) COMMERCIAL 'MOLECULAR KIT' and (b) 'DNA CHIPS' based applications for wildlife identification in forensics.